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1. What is RNasin® Plus RNase Inhibitor?

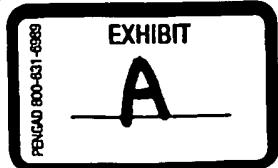
RNasin® Plus RNase Inhibitor is a recombinant mammalian RNase inhibitor expressed as a soluble protein in *E. coli*. Through natural amino acid diversity RNasin® Plus RNase has increased resistance to oxidation when compared to the human protein.

2. Will RNasin® Plus RNase Inhibitor work better than native or Recombinant RNasin® Ribonuclease Inhibitor?

RNasin® Plus RNase Inhibitor is naturally more resistant to oxidative stress, increasing overall efficacy. As an added feature RNasin® Plus displays continued inhibition at higher temperatures. Also, RNasin® Plus is expressed by *E. coli* as a soluble protein allowing easy purification by a combination of ion exchange and hydrophobic interaction chromatography. No direct affinity chromatography is required. This new process yields >90% pure protein with no *E. coli* RNase carryover. However, the mechanism of action remains the same for RNasin® Plus and native or Recombinant RNasin®. The inhibitory mechanism is an inhibition of eukaryotic RNases via stoichiometric 1:1 noncovalent binding of the RNasin® Plus RNase Inhibitor to an RNase.

3. How is RNasin® Plus RNase Inhibitor more resistant to oxidative stress than native or recombinant RNasin®?

Two cysteines in the human protein have been identified as especially sensitive and react by forming a disulfide that can block the active site of the inhibitor (1). RNasin® Plus RNase Inhibitor, through natural amino acid diversity, lacks the ability to form blocking disulfide.



4. What are the characteristics of RNasin® Plus RNase Inhibitor?

During development of the RNasin® Plus RNase Inhibitor Promega scientists discovered continued inhibition of RNases even above the normal denaturation temperature of the RNasin® Plus molecule. A mixture of RNasin® Plus and a pure RNase, like RNase A heated to at least 70°C for 15 minutes, and the RNase A activity does not return to normal levels when cooling to normal temperatures, such as those used in the RT step of RT-PCR. It has been demonstrated to work in this manner with a complex mixture of RNases in a rat liver protein extract (Sigma Cat.# L1380). Rat liver is known to contain ribonucleases (2). No detectable RNase activity, as determined by RT-PCR, is observed when a mixture of rat liver RNases and RNasin® Plus RNase Inhibitor is heated to 70°C for 15 minutes followed by the addition of 100ng or 10ng of template RNA and incubated for an additional hour at 37°C.

5. How can I use the characteristics of RNasin® Plus RNase Inhibitor to be protect my RNA template?

Many protocols, including those for ImProm-II™ Reverse Transcription System require an initial thermal denaturation of the RNA template of interest in the presence of reverse transcription primers for 5–10 minutes at 70°C followed by a quick chill step that denatures secondary structure in the RNA template, allowing greater sensitivity of PCR. In light of the new activities identified for RNasin® Plus RNase Inhibitor, it may now be added at this step to protect the RNA template during thermal denaturation. RNases that were present during the thermal denaturation will be inactivated; however, more RNase inhibitor should be added during full RT reaction assembly in the event additional exogenous RNases are inadvertently added to the reaction from pipetting components or other sources.

6. How do the characteristics of RNasin® Plus RNase Inhibitor relate to high temperature reverse transcriptase (RT) reactions?

The characteristics of RNasin® Plus RNase Inhibitor allow you to set up your high temperature first-strand synthesis reactions and take them to reverse transcript at reaction temperatures above 50°C. This gives researchers RNase protection when transcribing RNA templates with high secondary structure.

7. What applications are compatible with RNasin® Plus RNase Inhibitor?

RNasin® Plus RNase Inhibitor has been tested in RT-PCR and is compatible with enzymes such as AMV, M-MLV and ImProm-II™ Reverse Transcriptases or Taq and T7 DN Polymerases. RNasin® Plus RNase Inhibitor has also been tested and found to be compatible with quantitative, real-time RT-PCR reactions in a TaqMan® Assay. The new inhibitor is also compatible with the Riboprobe® System for in vitro transcription using the T3, T7 and T3/T7 RNA Polymerase. RNasin® Plus RNase Inhibitor can also be used with Wheat Germ Extract and Rabbit Reticulocyte Lysate for in vitro translation from an RNA template, as well as the TNT® Wheat Germ and TNT® Reticulocyte Lysate System for coupled in vitro transcription/translation.

References

1. Kim, B.M., Schultz, L.W. and Raines, R.T. (1999) Variants of ribonuclease inhibitor that resist oxidation. *Protein Sci.* **8**, 430–4.
2. Zhao, W. et al. (1998) Ribonucleases from rat and bovine liver: Purification, specificities and structural characterization. *Biochim. Biophys. Acta* **1384**, 55–65.